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14. ABSTRACT

Recurrent disease is the most daunting aspect of cancer therapy; however, how tumor cells become dormant and later recur is poorly understood. It is of paramount importance to decipher the underlining molecular mechanism of dormancy in order to define specific targets for the treatment of recurrent metastatic disease. The overall goal of this project is to identify key factor(s) and signaling that control tumor stem cell dormancy. In this fiscal year, we have established a unique model of tumor dormancy and recurrence in vivo using CSCs of prostate cancer cell line. This model provides us with an excellent tool to explore the molecular mechanism of tumor cell dormancy of CSCs in prostate cancer in bone. In the next fiscal year, we plan to define the roles of SPARC and Noggin in controlling bone microenvironment for dormancy and recurrence and we have already obtained approval of no-cost extension of this grant. We believe that the results of our experiments will provide important clues to identify specific targets for recurrent prostate tumor so that we can confine metastasized tumor to dormant state.

15. SUBJECT TERMS
Prostate cancer, tumor stem cells, dormancy, bone metastasis

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INTRODUCTION

Adenocarcinoma of the prostate is currently the most prevalent cancer in men in the United States and represents 36% of all cancers among men (1). It is estimated that more than 210,000 new cases are diagnosed and 32,000 patients succumb to this disease every year (1). Although patients with localized lesions can be cured by radical prostatectomy or radiotherapy, more than 90% of cancer deaths are attributed to metastatic disease (2). Even those patients who have localized cancer and have been "successfully" treated with surgery often experience "recurrent" disease after many years. How metastatic tumor cells become dormant is virtually unknown and it is of paramount importance to elucidate the underlining molecular mechanism of dormancy and recurrence, which may lead to identification of new therapeutic targets for recurrent disease. We hypothesize that a dormant cell is derived from tumor stem cells and maintains the original characteristics of stemness as well as the ability of recurrence and that factor(s) in the microenvironment determine the balance between dormancy and recurrence.

BODY

Task 1. To isolate tumor stem cells from prostate cancer patients and from metastatic tumor cell lines and screen "dormant" cells in animal followed by profile analysis of these cells

(a) We will first isolate tumor stem-like cells (CSCs) from patients and also from the prostate tumor cell line (PC3mm) and inject them into prostate of NOD/SCID mice to grow tumors. They will then be surgically removed and stem cells will be isolated again followed by labeling them with CMFD.

Progress

According to the cancer stem cell theory, a dormant and recurrent tumor cell must retain stem cell-like property (3,4). Therefore, we first isolated CSCs population ($CD24^-/CD44^+/CD133^+$) from PC3mm cell and injected them into mice. Our result of limited dilution analysis for tumor initiating ability indicates that the CSC population was significantly more tumorigenic than non-stem cells (Fig. 1). Aim 1a is in progress.

	Out growth/injection			Frequency of tumor-initiating cell (95% CI)
Cells injected	10^4	10^3	10^2	
Stem cell	5/6	3/6	1/6	1/3,181 ** (1/8,043-1/1,258)
Non stem	2/6	1/6	0/6	1/18,399 (1/59,298-1/5,709)

**P=0.00697

Fig. 1. Isolation of cancer stem-like cells (**A**) CSCs isolated from PC3mm were injected subcutaneously into nude mice, and the growth of tumor was monitored by bioluminescence imaging (BLI). The results of this limiting dilution analysis indicate that the CD24-/CD444+/CD133+ population has significantly more tumor initiating ability.

(b) The labeled stem cells will be injected into tibial bones of NOD/SCID mice and allowed to grow for 3 weeks. We will then isolate tumor cells from the bones and sort the cells into "first growing" and "slow growing" cell populations by FACS.

Progress

To establish a model to further study mechanisms of dormancy and recurrence, we isolated aggressively and poorly growing tumor cells after injecting CSCs of PC3mm into tibiae of mice and they were named Aggressive and Indolent cells. Interestingly, there was no significant difference in the growth and invasive ability between these cells in *in vitro* culture (Fig. 2A-B). Strikingly, however, when they were implanted into tibiae of mice, the growth rate was significantly different between these cells (Fig. 2C). Aim 1b is in progress.

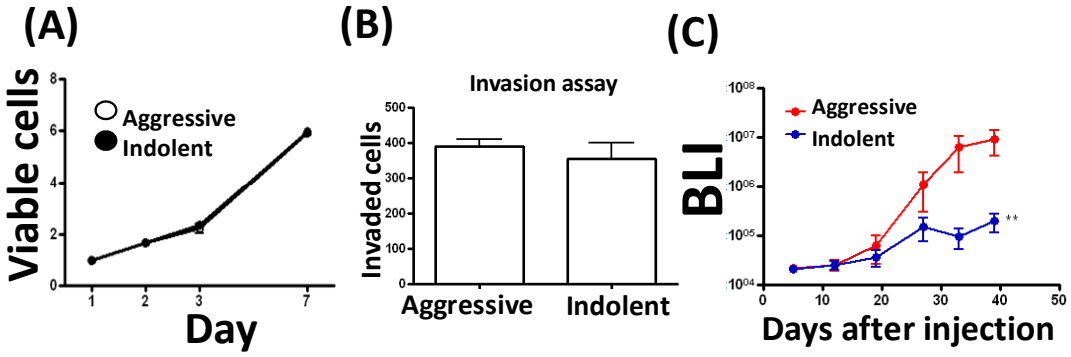

Fig. 2. Establishing a model of tumor dormancy and recurrence. (**A-B**) 1000 cells of CSCs from PC3mm were injected into nude mice tibiae and Indolent and Aggressive cells were isolated from tumors with slow and fast growth, respectively. The cell viability (A) and invasive ability (B) of Aggressive and Indolent cells was measured by the MTS assay and Matrigel transmigration assay in vitro. (**C**) Aggressive and Indolent PC3mm cells were injected into tibiae of nude mice, and the growth of tumor was monitored by bioluminescence imaging (BLI).

(c) We will examine the cells by two global expression analyses: (i) gene expression array of Affymetrix and (ii) antibody-based phospho-specific protein array.

Progress

To understand the molecular basis of the differences, we first performed Affymetrix microarray profiling analysis for these two cell lines and found that SPARC and Noggin were most significantly up- and down-regulated in Indolent cells, respectively (Fig.3A). These results were confirmed by qRT-PCR and Western blot (Fig. 3 B-D). Noggin is an inhibitor of BMPs, while SPARC is a matrix-associated protein and it has been reported to be involved in cell-cycle regulation. Interestingly, we found that SPARC had significant inverse correlation with metastasis of prostate cancer patients, using an existing GEO data (Fig. 3E). Aim 1c is in progress.

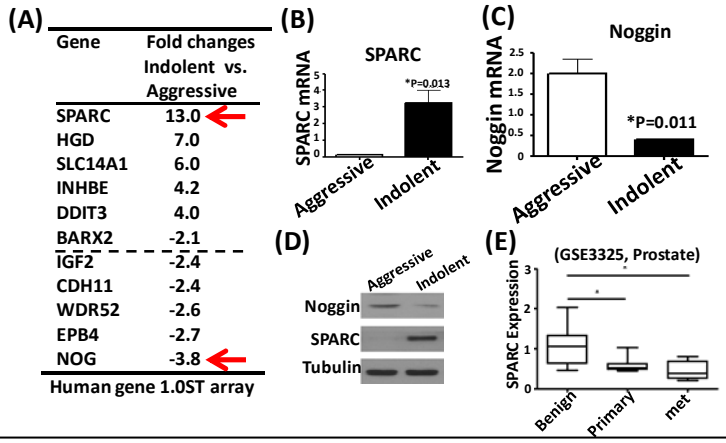

Fig. 3. Indolent cells express SPARC and Noggin. (**A**) Affymetrix expression array was performed for Aggressive and Indolent cells, and topmost 5 up- and down –regulated genes were listed. (**B,C,D**) The expression of SPARC and Noggin in Aggressive and Indolent cells was confirmed by qRT-PCR and Western Blot. (E) The expression of SPARC was examined in patient samples of prostate tumor with benign, primary and metastatic disease, using existing cohort data (GSE3325).

Task 2. To inject the dormant and non-dormant stem cells into the bone of mice and perform expression profile analysis for the "recurrent" tumor.

(a) We will inject the "fast growing" and the "slow growing" stem cells that are isolated in the first aim into the tibial bone of NOD/SCID mice and let them grow to form tumors. This recurrent tumor as well as "fast growing" tumor will be removed and expression profile as described in the first aim will be performed.

Aim 2a is in progress.

KEY RESEARCH ACCOMPLISHMENTS

1. We have isolated CSCs from prostate cancer cell line, PC3mm.
2. We have isolated Indolent and Aggressive cell lines as dormant and recurrence model.
3. We found that Dormant cells express SPARC and Noggin that may contribute to the dormancy.

REPORTABLE OUTCOMES

Peer reviewed publications

Aya Kobayashi, Sambad Sharma, Fei Xing, Puspa R. Pandey, Misako Watabe, Sudha K. Pai, Kounosuke Watabe. Roles of SPARC and Noggin in dormancy of prostate cancer stem-like cells in bone. Manuscript in preparation

Abstract/presentation

1. Aya Kobayashi, Hiroshi Okuda, Puspa Pandey, Misako Watabe, Sudha Pai, Fei Xing, Shigeru Hirota, Wen Liu and Kounosuke Watabe. Dormancy and recurrence of prostate cancer stem cell are regulated by bone morphogenetic protein 7 in bone. 2011 IMPaCT conference. Orlando, Fl.

Employment

1. Dr. Hiroshi Okuda (postdoc) has been partly supported by the current grant.
2. Dr. Megumi Iiizumi (postdoc) has been partly supported by the current grant.
3. Mr. Rabindra Karki (graduate student) has been supported by the current grant.

CONCLUSIONS

We have established an unique model of tumor dormancy and recurrence in vivo using CSCs of prostate cancer cell line. These two cell lines grow at a similar rate and there is no difference in their invasive ability. However, when they are transplanted into tibial bone in mice, Indolent cell stays as dormant and Aggressive cell rapidly grows and form tumors. Importantly, Indolent cell expresses significantly higher level of SPARC and Noggin compared to Aggressive cells. Therefore, this model provides us with an excellent tool to explore the molecular mechanism of tumor cell dormancy of CSCs in prostate cancer in bone.

So what?

We have developed a unique set of prostate cancer cell lines (Indolent and Aggressive variants) as a model system for dormancy and recurrence. To the best of our knowledge, this is the first set of

human prostate cell lines that recapitulate at least part of the process of tumor dormancy and recurrence. We hypothesize that high expression of SPARC and down-regulation of Noggin induces tumor cell dormancy, while down-regulation of SPARC and high expression of Noggin induces recurrent tumor growth in bone environment. Therefore, we plan to define the roles SPARC and Noggin in controlling bone microenvironment for dormancy and recurrence. We will test this hypothesis and pursue the rest of the tasks in the next fiscal year and we have already obtained approval of no-cost extension of this grant. The overall goal of this project is to identify key factor(s) and signaling that control tumor stem cell dormancy, particularly in the bone environment. We believe that the results of our experiments will provide important clues to identify specific targets for recurrent prostate tumor so that we can confine metastasized tumor to a dormant state.

REFERENCES

1. Cancer Facts & Figures (2009), American Cancer Society.
2. Henderson, B.E., Bernstein, L and Ross, R. (1997) Cancer: Principles and practice of oncology. Ed. Devita ,VT. pp219-257, Lippincott-Raven
3. Pantel, K., and C. Alix-Panabieres. (2007). The clinical significance of circulating tumor cells. *Nat Clin Prac Oncol*. 4:62-63.
4. Polyak, K., and R.A. Weinberg. (2009). Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nat Rev Cancer*. 9:265-73.